NOVEL, EFFICIENT SYSTEM FOR CULTURE OF TRANSDUCED HEMATOPOIETIC STEM PROGENITOR CELLS (HSPC) FOR VECTOR COPY NUMBER DETERMINATION

City of Hope

Laboratory for Cellular Medicine
Engineering Cures

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Objectives

- ✓ Develop a new method for culture of lentiviral transduced HSPC for determination of VCN, that is more consistent and less laborious.
- ✓ Compare the cell viability, cell expansion and phenotype of gene transduced HSPC (TDX-HSPC) cultured using G-Rex method versus Standard method.

Methods

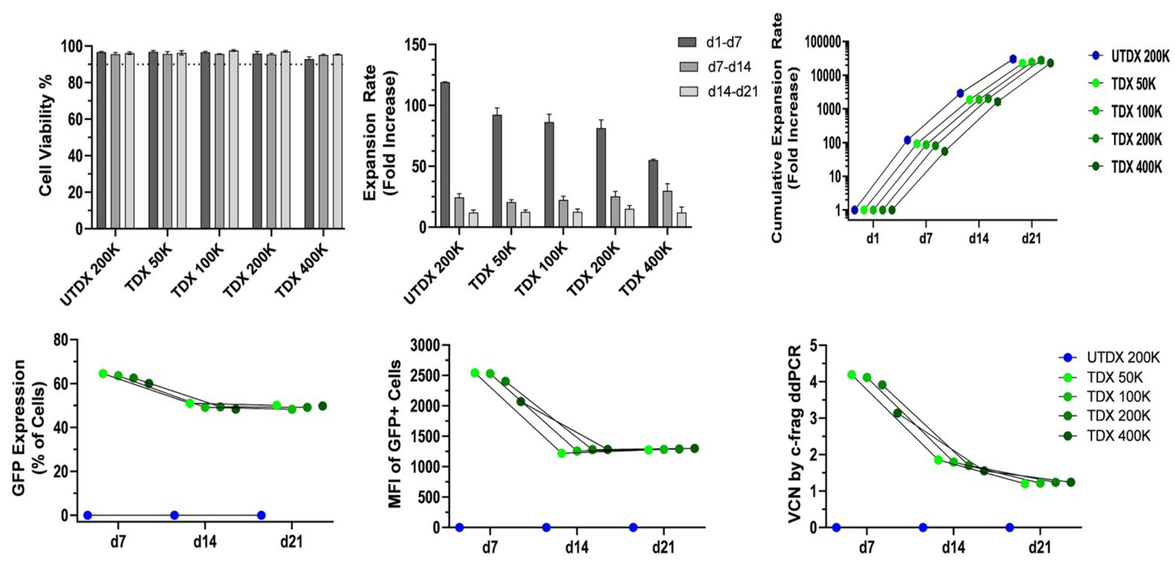
d0 CD34 Pre-Stim	d1 LVV Transduction	d2 Harvest	Liquid Culture (d1 – d14) 7-Cytokines; IMDM (20% FBS; SCF, GM-CSF, Flt3-L, Epo, IL-3, IL-6 & TPO)
N=3 Thawed CD34+ HSPC SFT3 StemSpan AOF 4E+06 cells/mL	LG631MGMT v2b MOI=20 SFT3 StemSpan AOF 2E+06 cells/mL UTDX CTRL	3 Washes Viability CD34 Flow Analysis CFU Assay Liquid Culture (VCN by ddPCR)	

Liquid Cultures		Standard Method	G-Rex Method
Seeding Conditions		12-Well Plate	24-Well G-Rex
D1 Post-TDX	Cell #	$2x10^5$	$2x10^5$
	Cells/mL	$1x10^5$	$2.5x10^4$
	Cells/cm^2	$2.6x10^4$	$1x10^5$
D7 Post-TDX	Cell #	$5x10^5$	$5x10^5$
	Cells/mL	$5x10^5$	$6.3x10^4$
	Cells/cm^2	$1.3x10^5$	$2.5x10^5$

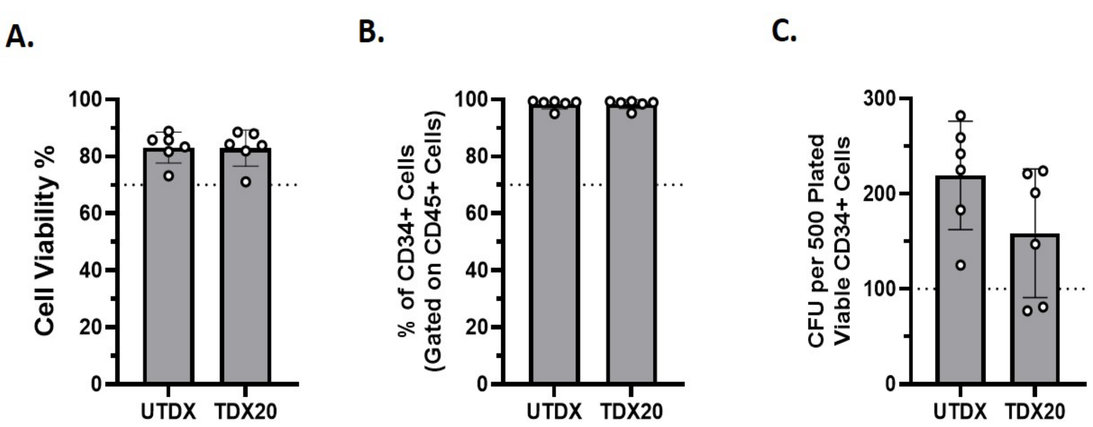
	12-Well Plate	24-Well G-Rex
Surface Area	3.8 cm^2	2 cm^2
Volume Capacity	6.9 mL	8 mL
Working Volume	2 mL	8 mL
Wells per Culture	Multiple	1
Limiting Factor	Nutrients and Oxygen	Membrane Surface of Well (Gas Permeable)
Maintenance	Triweekly	Weekly

Results

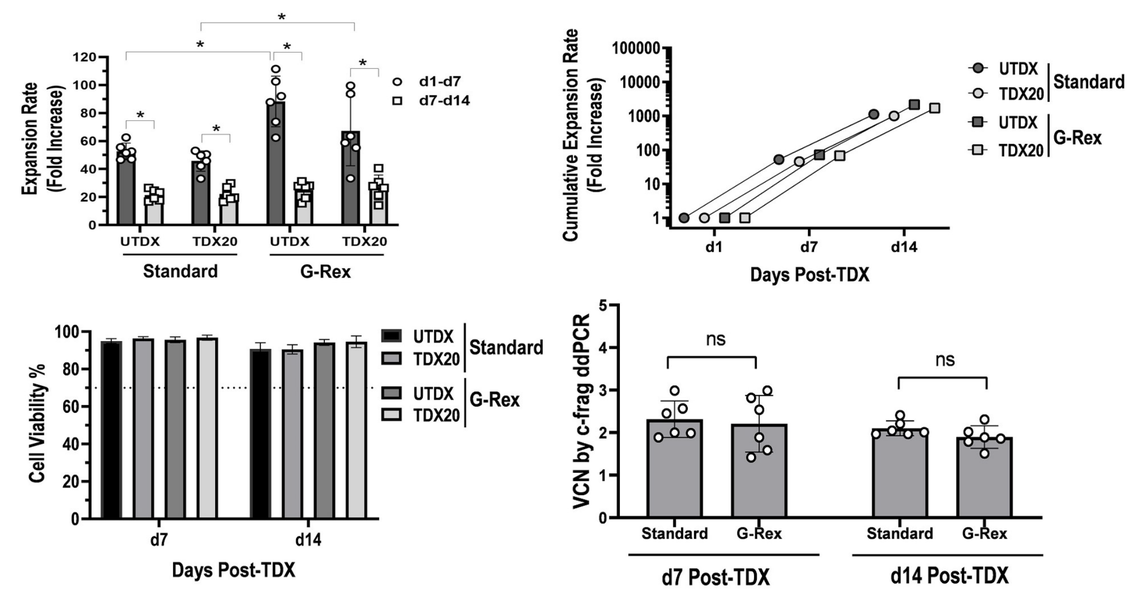
Establishing Seeding Conditions to culture HSPC in G-Rex Plates (TDX with GFP LVV)



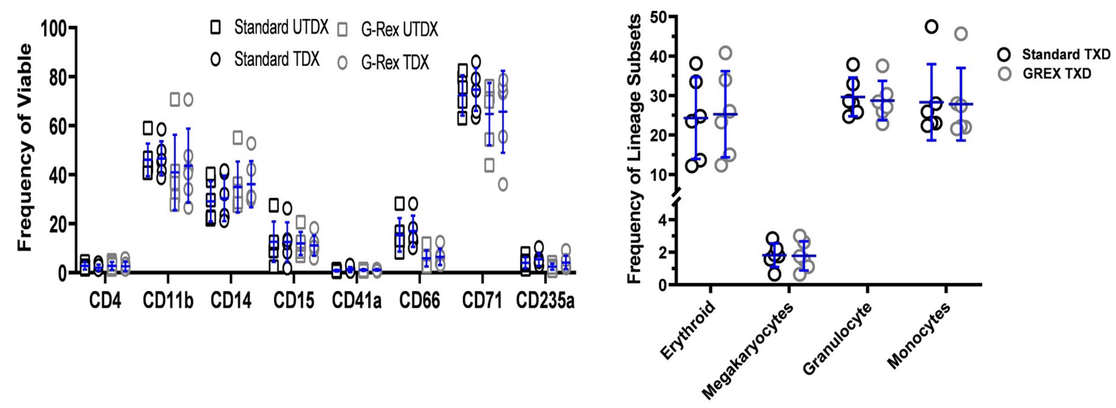
Characterization of TDX-HSPC (LG631b LVV; MOI=20)



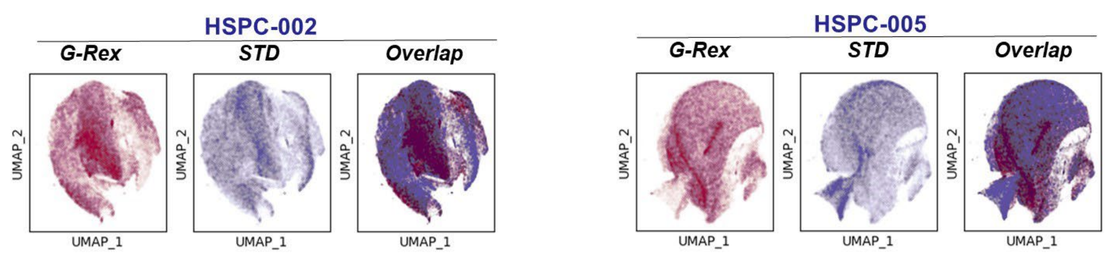
Expansion Rates and VCN of TDX-HSPC



Lineage Analyses of TDX-HSPC Cultures



UMAP Representation of Lineage Phenotypes



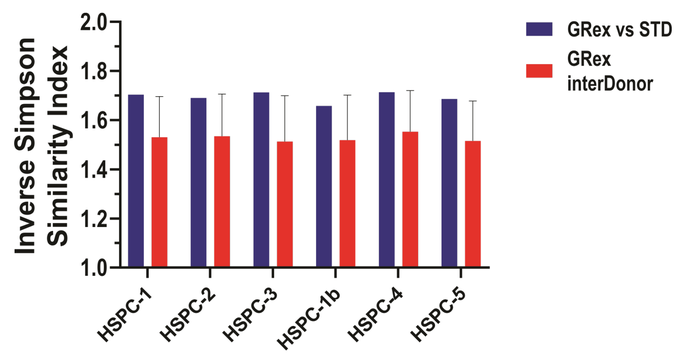
Red: UMAP of G-Rex data. Blue: UMAP of STD data. Overlap: UMAP of STD plot projected onto the UMAP of G-Rex plot

Similarity Analysis of Progeny of TDX-HSPC Cultures

Inversed Simpson Similarity Index (D)

$$D = 1/\sum_{s\in\{0,1\}} p_s^2$$

D=1, represents maximal diversity
D=2, represents maximal similarity



Conclusions / Future Directions

- ✓ **Hematopoietic Liquid Cultures in G-Rex support robust expansion and differentiation of TDX-HSPC, with transgene levels (VCN) similar to those observed using a Standard method.**
- ✓ **Hematopoietic lineage analyses of TDX-HSPC cultured in G-Rex showed no marked differences in comparison to the progeny of STD cultures. Overall similarity analysis using the inversed Simpson Index showed high similarity of d14 lineage phenotype between the two methods.**
- ✓ **The Cell Viability of G-Rex cultures was consistently high during culture (d7, 96.8%; d14, 94.6%) and comparable to that seen in Standard cultures.**
- ✓ **Higher cell expansion rate was observed in G-Rex method during the first week, with similar expansion rates in the second week of culture.**
- ✓ **This novel, G-Rex-based system of TDX-HSPC expansion/differentiation for VCN determination is simple and robust, providing marked labor and cost savings.**
- ✓ **This system is being integrated in the QC testing for release of TDX-HSPC Drug Products for clinical manufacturing. This method is suitable for automation using a robotic platform integrating genomic DNA preparation and ddPCR processing.**

Acknowledgments

CIRM 2.0 CALIFORNIA'S STEM CELL AGENCY

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